PRECISION METERING OF MICROLITER VOLUMES OF BIOLOGICAL FLUIDS IN MICRO-GRAVITY

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CO-INVESTIGATORS:	WT Norfleet, VD Kizzee (KRUG Life Sciences, Inc)
FLIGHT DATES:	May 3, 22, and 24, 1990

SUMMARY:

Eastman Kodak Company has served as a supplier of experimental diagnostic equipment for the SSF HMF, including a clinical chemistry analyzer. An important part of the clinical chemistry analysis process is introduction of a sample of the biological fluid of interest, usually whole blood, plasma or serum, to the sensor for the chemical constituent of interest. The potential difficulty associated with fluid handling in absence of gravity or in microgravity environments was recognized early in the process for design and development of a clinical chemistry analyzer useful in microgravity, but was incorporated only as a research topic within a contract for development of a medical development unit. Kodak and KRUG had discussed plans for KC135 experiments to investigate liquid fluid handling since 1986. Focus on this problem was offered by Kodak following requests by KRUG in 1989. During early 1990, a KC135 flight was scheduled and Kodak proceeded with experiment design and equipment fabrication in cooperation with KRUG. Conventional and experimental devices were planned to be tested. Experiments were designed that would test extreme cases of fluid and surface behavior anticipated for clinical chemistry system design and that would be able to be performed in the unusual and unstable laboratory setting of the KC135. The experiments aboard the KC135 were performed by Dr. Bill Norfleet (KRUG) with inflight assistance of Mr. Victor Kizzee (KRUG) and preflight instruction of Mr. Richard Columbus (Kodak), Dr. Harvey Palmer (Univ of Rochester) and Ms. Deborah Freyler (Kodak).

The experiments were able to be conducted generally as planned, but unplanned effects of cabin pressure changes during parabolic maneuvers confused results of initial automated pipet tests. Results of experiments showed that precision metering of microliter volumes of biological fluids is possible in microgravity environments and indicated that fluid handling is best addressed using developing integrated blood collection system technology. Further development and testing of discrete sample container technology using similar test protocol and addition of other processing steps is recommended. Video record of results is in preparation by Kodak.

Note: The primary record of results of these KC135 flight tests is video casset records obtained during the experiments aboard the KC135 aircraft. An edited record that focuses on important liquid transfer processes and demonstrations of proposed concepts is in preparation by Kodak. This record should be obtained and reviewed in cooperation with the principal investigators as the primary report of experiment results.

OBJECTIVE:

Demonstrate and investigate concepts for transferring accurately known and reproducible microliter volumes of biological fluids from sample containers onto dry chemistry slides in a microgravity environment. Compare specific liquid transfer tip designs. Obtain information for design of a liquid sample handling system to enable clinical chemical analysis in microgravity.

METHOD:

Disposable pipet tips and pipet devices that were designed to transfer microliter volumes of biological fluid from a (test tube) sample container onto dry chemistry slides in 1-g environment were used during micro-g periods of parabolic trajectories of the KC135 aircraft. The transfer process was recorded using charge couple device camera and video casset equipment. Three specific disposable pipet tip designs their function during micro-g exposure were compared. Metering behavior of water, a synthetic aqueous protein solution, and anticoagulated human whole blood was compared. Transfer of these liquids to 2 substrate materials representative of rapidly wettable and slowly wettable dry chemistry slide surfaces was compared. Checklist protocols were developed by Kodak to assist during preflight training, preparation for flights and in flight.

EQUIPMENT:

An electrically powered pipet, rigid holder, dry slide substrate material, camera, light, video recorder and power supplies were assembled on a mechanical plate and frame "breadboard" to be operated by a single experimenter and to be transported as a single unit. Micro-g compatible holders for liquid sample vials and pipet tips were also attached to the breadboard to be accessible to the experimenter/operator. The breadboard was attached to a workstation table with four bolts through the plywood table top; the table was anchored to the aircraft floor with tension straps over the table attached to anchor studs. A second video casset recorder (Sony hand held VHS) with a small flat screen monitor was attached to the table using Velcro so that the camera's close up view of the pipet tip and the liquid metering process was available for the experimenter/operator during the experiment.

Modification of the original equipment was made to incorporate an experimental liquid container and metering tip system on the second flight. The basic layout of pipet, camera, recorder and light were unchanged. A manually actuated pipet was used. The shape of the pipet tip was the same as one of the 3 previous designs. The third flight incorporated a modification of the pipet used for the first experiment, which permitted venting of the air column within the pipet between the liquid sample and solid bellows/piston to prevent displacement of the liquid in the pipet tip due to ambient pressure changes.

When actuated in the aspirate mode, the pipet would aspirate 110 microliters of liquid. When actuated in the dispense mode, the pipet would dispense a volume of 10 microliters.

[Note: A structural load analysis was prepared for the breadboard. The equipment, material and procedures were approved by NASA KC135 program personnel prior to the first experiment. Permission was obtained from NASA/SA/SLSD to use the 1-2 milliliter volume of human whole blood required for the experiments. Copies of Kodak's proposal for KC135 experiments, test plan and hazard analysis, structural analysis of the KC135 metering breadboard, and of a memo indicating permission from NASA/SA for use of human blood (plasma) in the experiment, are attached.]

PROCEDURE:

The experimenter/operator placed pipet tips on the pipet, filled pipet tips from the sample vial, positioned substrate under the pipet tip, and pressed a button to actuate the pipet to dispense liquid onto the substrate. For certain experiments, a manually actuated pipet was used. Placing and filling pipet tips and placing a substrate strip mounted on a plate under the pipet on a slide rail was done prior to a set of 10 parabolic maneuvers. After each parabola and pipet actuation/liquid dispense process, the substrate was advanced one detent lock position on the slide rail. A pipet tip was replaced and filled and a substrate strip was replaced on the slide rail following completion of 10 parabolic maneuvers.

RESULTS:

The function of the electrically powered pipet and different pipet tip designs to transfer microliter volumes of liquid onto slide substrate material during micro-g was recorded on video casset, which is in preparation by Kodak. Observations of the experimenter/operator, Dr. Norfleet, are described below:

Flight 1 (May 3, 1990):

Function of the pipet to meter accurate volumes of fluid was affected by changes in cabin pressure of the aircraft as engine power was changed during parabolic maneuvers. The change in ambient pressure caused the liquid column in the pipet tips to change position either up or down, with the result that accurate volumes of fluid were not transferred onto the substrate material. An important finding was demonstration of the ability to make and break a column of liquid between the pipet tip and the slide substrate in micro-g. A concern of principal investigators was that fluid columns would form but would not break in micro-g: Once a column formed between the tip and the substrate, all of the liquid in the pipet tip might be drawn from the pipet tip onto the substrate or to the extent that the substrate became saturated. Columns of water and protein solutions were shown to form and break during micro-g periods.

Flight 2 (May 22, 1990):

The experimental liquid container and metering system was used and functioned well during the flight to contain the blood sample and to meter

accurate volumes onto the slide substrate. The experimental container was filled with anticoagulated whole blood prior to the flight. The metering device was a manually operated volumetric pipet which contacted the container at a metering port. The design of the tip to transfer fluid from the container to the substrate used concentric ring edges in "stair step" arrangement to limit and direct fluid motion from the pipet tip onto the slide substrate. Accurately directed, reproducible volumes of blood appeared to transfer from the container onto the substrate on demand. There were no obvious problems handling and dispensing small blood volumes in micro-g using this system.

Flight 3 (May 24, 1990):

The modified pipet from flight 1, modified to vent during cabin pressure changes to equalize pressure above and below the liquid column in the end of the pipet tip, did not seem to solve the problem encountered on flight 1. No significant difference was noted, indicating that venting was ineffective or that other effects, e.g. surface tension, were dominant. Ability to form and break fluid columns in micro-g was confirmed.

CONCLUSIONS:

The basic mechanism of a pipet that displaces air to displace the liquid of interest from a pipet tip can function to transfer an accurate, microliter volume of blood, plasma or water liquid in micro-g. Liquid reservoir and dispense methods, however, may require special design to limit liquid volume transferred or/and pressure capacitance effects. These liquid transfer functions, which are normally performed in a clinical laboratory work bench setting with stable work bench and generous work area, can also be performed in suboptimal settings such as the KC135 aircraft with rapidly changing ambient pressure, temperature and gravitational acceleration at the work station. The experimental container that was specially designed to maintain an integral liquid column within the container and an extended liquid column length functioned well to transfer accurate, limited volumes of blood. Conventional pipet designs did not perform well to meter accurate liquid volumes. Formation and breakage of liquid columns (or bridges) between pipet tips and slide substrates, occurred in microgravity, whether the liquid was a protein solution, blood or water and whether the substrate was readily or slowly wettable. Transfer of accurate, microliter liquid volumes is possible in micro-g environments.

Analysis of video data by Kodak and may provide more information regarding transfer processes for blood, plasma, serum and possibly other biological fluids in micro-g.

RECOMMENDATIONS:

The integrated blood (biological fluid) collection, processing and metering system should receive further development and test. Rate of liquid metering and length of the liquid column within the sample container might be compared with metered volume accuracy in micro-g. A similar test protocol should be used and should add other necessary liquid handling or processing steps that are needed for laboratory diagnostic procedures in micro-g environments, e.g. preparation of quality control fluids.

Attachments:

Proposal for KC135 experiments: Testing strategies for metering microliter volumes of biological fluids in microgravity, RL Columbus and HJ Palmer.

Test plan for KC135 experiments: Precision metering of microliter volumes of biological fluids in microgravity (including hazard analysis/safety certification considerations and equipment sketches).

Supplement to test plan for KC135 experiments (description of experiment 4 to include use of human blood plasma).

Structural analysis of KC135 (liquid) metering breadboard. (letter and attachments from Kodak/J. Quenin to KRUG/B. McKinley, April 7, 1990.)

Memorandum for record, re: use of human blood plasma in proposed precision fluid metering experiments aboard the KC135, April 25, 1990.

NASA PHOTO REFERENCE

S90-39608
Setup of the experiment workstation

S90-39611
System for delivery of volumes

S90-39775
Preparing a sample

S90-39780 - 82
Performing various steps in monitoring the instruments